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Scope of Research

Our research covers the comprehensive understanding of the physiological roles of biocatalysts (enzymes) as well as the reaction mechanism, the structure and properties of each enzyme. 1) Development of intermediate analogue inhibitors of acyl-activating enzyme superfamily that plays pivotal roles in plant hormone homeostasis and secondary metabolite biosynthesis of plants. 2) Design and synthesis of transition-state analogue and mechanism-based inhibitors of γ -glutamylcysteine synthetase and γ -glutamyl transpeptidase, the key enzymes in glutathione biosynthesis and its metabolism, respectively. 3) Studies on structural basis for bioluminescence change of firefly luciferase. 4) Studies on the activation/inactivation process of plant hormones. 5) Molecular mechanism of regulation of coumarin biosynthesis in plants.

Research Activities (Year 2008)

Publications

Wada K, Hiratake J, Irie M, Okada T, Yamada C, Kumagai H, Suzuki H, Fukuyama K: Crystal Structures of *Escherichia coli* γ -Glutamyltranspeptidase in Complex with Azaserine and Acivicin: Novel Mechanistic Implication for Inhibition by Glutamine Antagonists, *J. Mol. Biol.*, **380**, 361-372 (2008).

Saino H, Mizutani M, Hiratake J, Sakata K: Biochemical Characterization of β -Primeverosidase—Expression with a Baculovirus Insect Cell System and Affinity Purification with a Primeverosylamidase as a Ligand—, *Biosci. Biotechnol. Biochem.*, **72**, 376-383 (2008).

Kai K, Mizutani M, Kawamura N, Yamamoto R, Tamai M, Yamaguchi H, Sakata K, Shimizu, B: Scopoletin is Biosynthesized via *ortho*-Hydroxylation of Feruloyl-CoA by an 2-Oxoglutarate Dependent Dioxygenase in *Arabidopsis thaliana*, *Plant J.*, **55**, 989-999 (2008).

Seki H, Ohyama K, Sawai S, Mizutani M, Ohnishi T, Sudo H, Akashi T, Aoki T, Saito K, Muranaka T: Licorice β -Amyrin 11-Oxidase, a Cytochrome P450 with a Key

Role in the Biosynthesis of the Triterpene Sweetener Glycyrrhizin, *Proc Natl Acad Sci USA.*, **105**, 14204- 14209 (2008).

Presentations

Rational Design of Specific Inhibitors of γ -Glutamyl Transpeptidase (GGT) and γ -Glutamylcysteine Synthetase for Modulating Cellular Glutathione Redox Status, Hiratake J, 2nd World Conference on Magic Bullets (Ehrlich II), Nuernberg, Germany, 4 October 2008.

Biochemical Characterization of Cytochrome P450 Monooxygenases in Plant Steroid Metabolism, Mizutani M, 7th Japan-US Seminar, Biosynthesis of Natural Products, “Enzymology, Structural Biology, and Drug Discovery”, San Diego, USA, 24 June 2008.

New Functions of P450s in Brassinosteroid Biosynthesis and Catabolism, Mizutani M, 9th International Symposium on Cytochrome P450 Biodiversity and Biotechnology, Nice, France, 10 June 2008.

Molecular Design and Synthesis of γ -Glutamylcysteine Synthetase Inhibitors

γ -Glutamylcysteine synthetase (GCS) catalyzes the ATP-dependent coupling of L-Glu and L-Cys to make γ -Glu-Cys, the first and the rate-limiting step in glutathione biosynthesis. Therefore GCS is an extremely important enzyme that controls the cellular redox status and detoxification potential through affecting the glutathione level and confers the cells with resistance against toxic xenobiotics such as reactive oxygen species and anticancer drugs. We designed and synthesized the sulfoximine-based transition-state analogue inhibitors **1a** and **b** with an emphasis on the recognition of the side chain of Cys by the enzyme [Figure 1. (a)]. The X-ray crystallographic studies on *E. coli* GCS indicated that the side chain of Cys was recognized by Arg132 [Figure 1. (b)]. The inhibitor **1b** with a cyano group at the side chain of Cys moiety was ca. 5 times more potent than the inhibitor **1a** with a methyl group, suggesting that the cyano group mimicked the SH of Cys to interact with the guanidino group of Arg132. The cyano sulfoximine **1b** was more than 6000 times as potent as buthionine sulfoximine (BSO), a most frequently used GCS inhibitor, thus serving as a new lead for effective drug for controlling the cellular glutathione biosynthesis.

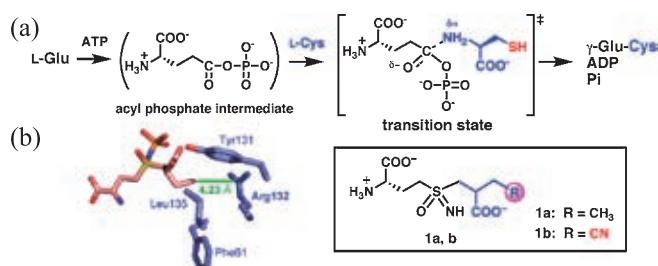


Figure 1. (a) The reaction mechanism of GCS and the sulfoximine-based transition-state analogue inhibitors **1a** and **1b**. (b) The X-ray structure showing the interaction with the side chain of Cys and Arg132.

Cytochrome P450s in Brassinosteroid Biosynthesis

Brassinosteroids (BRs) are plant steroid hormones that are essential for normal growth and development in plants. Cytochrome P450 monooxygenases (P450s) play crucial roles in BR biosynthesis, in which many oxygenations at steroidal skeleton and side-chain structure occur. Recent molecular genetic studies for BR-deficient mutants of Arabidopsis, rice, tomato, and garden pea have identified several P450 genes (*CYP85A*, *90A*, *90B*, *90C*, *90D*, and *724B*) so far. However, the catalytic functions of them remained ambiguous due to lack of biochemical study. Recently, we succeeded in functional expression of these P450s in a baculovirus-insect cell system as well as in a bacterial expression system, and their catalytic activities were determined in an *in vitro* assay. We found that *CYP90B* and *CYP724B* are redundant C-22 hydroxylases and also that *CYP90C* and *CYP90D* are redundant C-23 hydroxylases. *CYP90A* was found to catalyze C-3 oxidation and isomerization of 22-hydroxycampesterol and 22,23-dihydroxycampesterol to produce their corresponding 4-en-3-one. In contrast, campesterol is not metabolized by *CYP90A* at all. Taken together, we have proposed the campestanol-independent pathway of BR biosynthesis, which predominantly converts campesterol to 22-hydroxycampesterol and (22*S*,24*R*)-22-hydroxyergost-4-en-3-one to form bioactive BRs, without going through campestanol.

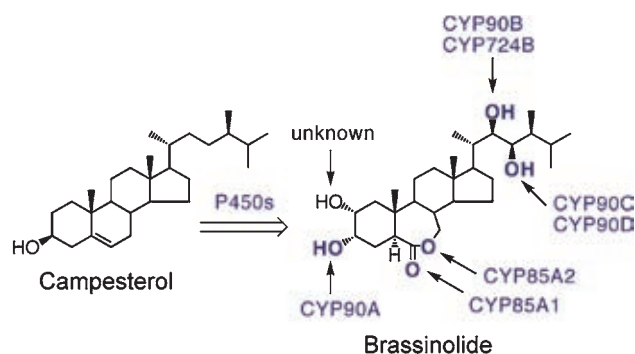


Figure 2. Cytochrome P450s in Brassinosteroid Biosynthesis CYP90A, C3-oxidase; CYP90B and CYP724B, C22-hydroxylase; CYP90C and CYP90D, C23-hydroxylase; CYP85A, C6-oxidase.

Grants

Hiratake J, Development of Chemicals to Control Glutathione Metabolism and Oxidative Stress for Use in Chemical Biology, Grant-in-Aid for Scientific Research

(B) (2), 1 April 2007–31 March 2010.

Mizutani M, Construction of Plant Oxygenase Library and Its Functional Characterization, Grant-in-Aid for Scientific Research (C) (2), 1 April 2006–31 March 2008.